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Filed:

09/513,362
February 25, 2000

REMARKS

Claims 1-30 are pending. Claims 1, 10 and 18 have been amended. Support for the amendment of claims 1, 10 and 18 can be found on page 13, line 37; page 30, lines 16-34; page 31, lines 5-36. The specification has been amended to correct a typographical error. Claim 4 has been amended for clarity. Claim 31 and 32 have been newly added. Support for newly added claims 31 and 32 can be found at page 38, lines 21-29. A version showing changes made is attached for the Examiner's convenience. An appendix of pending claims is also attached for the Examiner's convenience.

Priority

Applicants have amended the specification to correct for a typographical error and replace an unrelated application number with the correct related application number. Applicants thank the Examiner for noticing the error.

Rejections based under 35 U.S.C. § 103(a)

Claims 1-4, 6-10, 12-17 and 22-27 are rejected under 35 U.S.C 103(a) as being unpatentable over Navot et al. (U.S. Patent No. 6,335,165 B1) and Walt et al. (U.S. Patent No. 6,327,410 B1).

Navot et al. is directed to methods and kits for characterizing G-C rich regions of a nucleic acid of interest. Methods steps include modifying the guanine and cytosine residues by replacing them with residues complementary to adenine and thymine; amplifying the modified nucleic acid of interest and then subjecting the modified nucleic acid to sequencing which can include gel electrophoresis-free pyrosequencing. Navot et al. does not teach or suggest the use of

microspheres, distributed on the surface of a substrate. Navot et al. also does not teach or suggest kits which contain microspheres distributed on the surface of a substrate.

Walt et al. is directed to a microsphere-based analytical chemistry system and methods for making the same in which microspheres carrying bioactive agents may be combined randomly or in an ordered fashion and distributed on the surface of a substrate for detecting the presence or absence of target analytes in a sample through the use of an optical encoding scheme. Walt et al. does not teach or disclose pyrosequencing. Walt et al. is also silent with respect to pyrosequencing on microspheres.

In contrast, claim 1, (from which claims 2-4, 6-9, 22-23 and 26-27 depend), is directed to a method of sequencing a plurality of target nucleic acids by hybridizing sequencing primers to target sequences forming hybridization complexes that are attached to microspheres that are distributed on a surface of a substrate. The method further includes simultaneously extending the primers by the addition of nucleotides and finally detecting the release of pyrophosphate to determine the type of nucleotide added onto the primers. Claim 10 (from which 12-17, and 24-27 depend), is directed to a method of sequencing a plurality of target nucleic acids by providing hybridization complexes comprising target sequences and sequencing primers covalently attached to microspheres distributed on a surface of a substrate. The method further includes determining the identity of a plurality of bases at target positions by detecting the release of pyrophosphate to determine the type of nucleotide added onto the primers.

To establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. In addition,

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the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991).

The Examiner states that it would have been obvious to one of ordinary skill in the art to use the microspheres randomly distributed on the surface of Walt et al. as the beads in the pyrosequencing of Navot et al. The Examiner goes on to state that Walt et al. expressly provides the motivation to do so by stating that the synthesis of nucleic acids is separated from their placement on the array and random distribution of beads is fast and inexpensive. Applicants respectfully disagree.

Applicants submit that the Examiner's statement of the motivation expressly provided by Walt that the synthesis of nucleic acids can be separated from their placement on the array and random distributed of beads is fast and expensive, does not provide the specific guidance required to provide motivation to modify or combine the references of Navot and Walt to reach the claims of the present invention. The Examiner further cites to Walt, stating that the array in Walt can be used for sequencing (column 24, lines 51-52). See office action at page 5. The Applicants note that at column 24, lines 51-52, the actual statement in Walt is that the probes in the array are used for "sequencing by hybridization". Sequencing by hybridization is distinct from pyrosequencing. As stated above, Walt is silent with respect to pyrosequencing on a microsphere array.

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"Obvious to try" is not the standard and the very general statement that the Examiner points to in Walt et al. that the synthesis of nucleic acids can be separated from their placement on the array and random distribution of beads is fast and expensive, could apply to any number of methodologies. It is improper to use an obvious to try approach or to cite to only general guidance as to the particular form of the claimed invention or how to achieve it. See *In re O'Farrell*, 853 F. 2d 894,903, 7 USPQ2d 1673,1681 (Fed. Cir. 1988). It is insufficient that the prior art disclosed the components of the patented invention, either separately or used in other combination; there must be some teaching, suggestion, or incentive to make the combination made by the inventor. *Northern Telecom, Inc. v. Datapoint Corp.*, 908 F.2d 931, 15 USPQ2d 1321 (Fed. Cir. 1990). The district court did not commit reversible error "by requiring that a claimed invention be "clearly suggested" by the prior art in order to be obvious. *Gillette Co. v. S.C. Johnson & Son, Inc.*, 919 F.2d 720, 16 USPQ2d 1923 (Fed. Cir. 1990).

Applicants submit that there is lacking any specific guidance as to pyrosequencing on microspheres distributed on the surface of a substrate. In addition, there is lacking any suggestion in the references to modify the references or to combine them to reach the claims of the present invention. Therefore, the requirement that there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings has not been met. Accordingly, the rejection is improper and the Applicants respectfully request the withdrawal of the rejection.

Claims 5 and 11 are rejected under 35 U.S.C. 103(a) as being unpatentable over Navot et al. and Walt et al. as applied to claims 1 and 10, and further in view of Balch et al. (U.S. Patent No. 6,083,763). Basically, the Examiner's position appears to be that it would have been obvious

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to one of ordinary skill in the art at the time to combine the adapter probes of Balch et al. with the methods of Navot et al. and Walt et al. Applicants respectfully traverse.

The distinctions between Navot et al. and Walt et al. and the claims of the present invention are discussed above. As the Examiner points out, neither Navot et al. nor Walt et al. teach adapter probes.

Balch et al. is directed to a multiplexed molecular analysis system of detecting target analytes within a sample through the use of "charged coupled device" (CCD) technology. Balch et al. does not teach microspheres distributed on the surface of a substrate nor does it teach the use of pyrosequencing of nucleic acids.

In contrast, claim 5 is drawn to a method of sequencing nucleic acids by detecting the release of pyrophosphate to determine the type of nucleotide added to a sequencing primer by providing hybridization complexes attached through the use of capture and adapter probes to microspheres distributed on the surface of a substrate. Claim 11 is drawn to a method of sequencing a nucleic acid by determining the identity of a plurality of bases by detecting the release of pyrophosphate to determine the type of nucleotide added to a sequencing primer through the use of an adapter probe and a sequencing primer covalently attached to microspheres randomly distributed on a surface.

As stated above, to establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. In addition, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination

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and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, Supra

The Examiner states that it would have been obvious to one of ordinary skill in the art to have used the adapter probes of Balch for the formation of primer-target complexes in the combined method of Navot et al. and Walt et al. The Examiner states that the motivation to do so would have been that adapter probes delivered a unique binding domain for each site on an array. Applicants respectfully disagree.

In the instant case, there is lacking any suggestion or motivation to modify the references or combine reference teachings. As noted briefly above, the Examiner suggests that one of skill in the art would have been motivated to combine references because it was obvious to use the adapter probes of Balch for the formation of primer-target complexes in the combined method of Navot et al. and Walt et al. However, Applicants submit that this is a legally incorrect determination of motivation. The mere fact that references can be combined or modified does not render the resultant combination obvious unless the prior art also suggests the desirability of the combination. *In re Mills*, 916 F 2d 680, 16 USPQ2d 1430 (Fed. Cir. 1990).

There is no suggestion in any of the references of modifying or combining the references to reach the claims of the present invention. That is, while Walt et al. describes the use of microspheres for detecting the presence or absence of a target, there is nothing cited to in the references that would have motivated one of skill in the art to combine the adapter probes of Balch et al. with the microspheres of Walt and the pyrosequencing method of Navot et al. As stated above, it is insufficient that the prior art disclosed the components of the patented invention, either separately or used in other combination; there must be some teaching, suggestion, or incentive to make the combination made by the inventor. *Northern Telecom, Inc.*

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v. Datapoint Corp., 908 F.2d 931, 15 USPQ2d 1321 (Fed. Cir. 1990). The district court did not commit reversible error "by requiring that a claimed invention be "clearly suggested" by the prior art in order to be obvious. *Gillette Co. v. S.C. Johnson & Son, Inc.*, 919 F.2d 720, 16 USPQ2d 1923 (Fed. Cir. 1990). Here there is no suggestion in the prior art to reach the claimed invention much less a clear suggestion.

In addition, the Examiner's attention is respectfully drawn to *In re Lee*, 61 USPQ2d 1430 (CA FC 2002). In this case, the Examiner rejected the claims under 35 U.S.C. §103 and stated that the required motivation "would be that the automatic demonstration mode is user friendly and it functions as a tutorial". *Id* at 1435. The Federal Circuit stated that "deficiencies of the cited references cannot be remedied by the Board's general conclusions about what is "basic knowledge" or "common sense"". The Board's finding must extend to all material facts and must be documented on the record, lest the "haze of so-called expertise" acquire insulation from accountability. "Common knowledge" and "common sense", even if assumed to derived from the agency's expertise, do not substitute for authority when the law requires authority." (citing *In re Zurko*, 59 USPQ2d 1693 (CA FC 2001); see *Lee*, 1434-1435). In the present case Applicants submit that the Examiner has failed to point to anything specific in the cited references that would suggest or provide the motivation to combine the references or to modify them. The Examiner has also failed to document on the record what the common knowledge consists of by pointing to specifics and this is legally incorrect under *In re Lee*.

In this case, the Examiner has essentially used impermissible hindsight and "common sense" to conclude that the combination of these references would have been motivated by "adapter probes delivering a unique binding domain for each site on an array". This is legally incorrect under the Federal Circuit's analysis.

In conclusion, there is lacking a any suggestion in the prior art for combining the references to reach the claims of the present invention. Accordingly, the rejection is improper and the Applicants respectfully request the withdrawal of the rejection.

Claims 18-21 and 28-30 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Navot et al. and Walt et al. as applied to claims 1 and 10 above, and further in view of Nyren et al. (WO 98/13523). The Examiner's position appears to be that it would be obvious to one of ordinary skill in the art to combine the kits of Nyren to a kit and composition disclosed by Navot and Walt because kits are conventional in the field and provide benefits of convenience and cost-effectiveness. Applicants respectfully traverse.

The distinctions between Navot et al. and Walt et al. and the claims of the present invention are discussed above.

Nyren et al. discloses a method of sequencing DNA based on the detection of base incorporation by the release of pyrophosphate (pyrosequencing). Nyren et al. does not teach or suggest a method of pyrosequencing through the use of microspheres distributed on the surface of a substrate. Furthermore, Nyren et al. does not disclose kits which contain all the reagents necessary for sequencing on microspheres distributed on the surface of a substrate.

Claims 18-21 are drawn to kits for nucleic acid sequencing comprising microspheres distributed on the surface of a substrate, extension enzymes and labeled dNTPs.

As stated above, to establish a prima facie case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. In addition, the prior art reference (or references when combined) must teach or

suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, Supra.

The Examiner states that it would have been obvious to one of ordinary skill in the art to add the kits of Nyren et al. to a kit and composition disclosed by Navot et al. and Walt et al. because kits are conventional in the field of molecular biology and provide benefits of convenience and cost-effectiveness. Applicants respectfully traverse.

Here, neither Navot, Walt nor Nyren teach or suggest the use of kits containing a composition comprising a substrate with a surface comprising discrete sites and a population of microspheres randomly distributed on the sites.

There is lacking any suggestion or motivation to modify or combine reference teachings to reach the claims of the present invention.

As stated above, in *In re Lee*, "Common knowledge" and "common sense", even if assumed to derived from the agency's expertise, do not substitute for authority when the law requires authority." (citing *In re Zurko*, 59 USPQ2d 1693 (CA FC 2001); see *Lee*, 1434-1435). Accordingly, the Examiner's statement of the general benefits of convenience and cost-effectiveness is insufficient motivation to combine or modify the cited prior art references to reach the claims of the present invention.

In the present case Applicants submit that the Examiner has failed to point to anything specific in the cited references that would suggest or provide the motivation to combine the references or to modify them. The Examiner has also failed to document on the record what the common knowledge consists of by pointing to specifics and this is legally incorrect under *In re Lee*.

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In this case, the Examiner has essentially used impermissible hindsight and "common sense" to conclude that the combination of these two references would have been motivated by "the obvious benefits of convenience and cost effectiveness". This is legally incorrect under the Federal Circuit's analysis.

In conclusion, there is not substantial evidence as to the reason the skilled artisan, with no knowledge of the claimed invention, would have selected these components for combination in the manner claimed. A rejection cannot be predicated on the mere identification in the prior art of individual components of claim limitations. *In re Kotzab*, 55USPQ2d 1313, 1317.

Accordingly, the requirement that there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings has not been met. Accordingly, the rejection is improper and Applicants respectfully request the withdrawal of the rejection.

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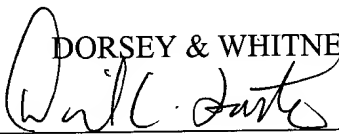
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CONCLUSION

Applicants submit that the claims are now in condition for allowance and early notification to that effect is respectfully requested. If the Examiner feels there are further unresolved issues, the Examiner is respectfully requested to phone the undersigned at (415) 781-1989.

Respectfully submitted,

Dated: 2/20/2003

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VERSION SHOWING CHANGES

IN THE CLAIMS

1. (amended) A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) provide an array comprising:

i) a substrate with a surface comprising discrete sites; and

ii) a population of microspheres comprising at least first and second

subpopulations, distributed at discrete sites on a surface of a substrate;

[a) providing first and second hybridization complexes comprising first and second target sequences, respectively and first and second sequencing primers, respectively, that hybridize to the first domain of said first and second target sequences, respectively, said first and second hybridization complexes attached to first and second microspheres, respectively, randomly distributed on a surface of a substrate]

b) providing a first hybridization complex comprising said first domain of a first target sequence and a first sequence primer, wherein said first hybridization complex is attached to said first subpopulation;

c) providing second hybridization complex comprising said second domain of a second target sequence and a second sequence primer, wherein said second hybridization complex is attached to said second subpopulation;

d) [b)] simultaneously extending said first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primer, respectively;

e) [c)] detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second primers, respectively; and

f) determining the identity and location of each microsphere.

4. (amended) A method according to claim 1 wherein said first and second hybridization complexes comprise:

a) said first and second target sequences [,respectively];

b) said first and second sequencing primers[,respectively, and];

c) first and second capture probes, [respectively] wherein said capture probes are covalently attached to said first and second microspheres, respectively.

10. A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

[a) providing first and second hybridization complexes a hybridization complex comprising first and second target sequences [said target nucleic acid and a capture probe covalently attached to a microsphere randomly distributed on a surface of a substrate], respectively and first and second sequencing primers, respectively, that hybridize to the first domain of said first and second target sequences, respectively, said sequencing primers covalently attached to microspheres randomly distributed on a surface of a substrate, wherein said sequencing primers microspheres comprise an identifier binding ligand that will bind a decoder binding ligand such that the identity and location of each microsphere can be determined;

b) determining the identity of a plurality of bases at said target positions, wherein said determining comprises simultaneously extending said first and second sequencing primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively;

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second sequencing primers, respectively.]

a) a) providing first hybridization complex comprising a first target sequence and a first sequencing primer that will hybridize to the first domain of said first target sequence,

b) providing a second hybridization complex comprising a second target sequence and a second sequencing primer that will hybridize to the second domain of said second target sequence, wherein said first and second sequencing primers are covalently attached to microspheres distributed on a surface of a substrate;

b) determining the identity of a plurality of bases at said target positions, wherein said determining comprises simultaneously extending said first and second sequencing primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primers, respectively; and

c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second sequencing primers, respectively.

18. A kit for nucleic acid sequencing comprising:

a) a composition comprising:

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- i) a substrate with a surface comprising discrete sites; and
- ii) a population of microspheres [randomly] distributed on said sites; wherein said microspheres comprise capture probes;
- b) a first extension enzyme; and
- c) dNTPs.

IN THE SPECIFICATION

This application is a continuing application of 09/425,633, filed October 22, 1999 and claims the benefit of priority of U.S.S.N.s 60/130,089 filed April 20, 1999; 60/135,051, filed May 20, 1999; 60/135,053, filed May 20, 1999; 60/135,123, filed May 20, 1999; [60/160,027] 60/160,927 filed October 22, 1999; 60/161,148 filed October 22, 1999 and 60/160,917, filed October 22, 1999.

PENDING CLAIMS

1. A method of sequencing a plurality of target nucleic acids each comprising a first domain and an adjacent second domain, said second domain comprising a plurality of target positions, said method comprising:

a) provide an array comprising:

- i) a substrate with a surface comprising discrete sites; and
- ii) a population of microspheres comprising at least first and second subpopulations, distributed at discrete sites on a surface of a substrate;

b) providing a first hybridization complex comprising said first domain of a first target sequence and a first sequence primer, wherein said first hybridization complex is attached to a first microsphere;

c) providing second hybridization complex comprising said second domain of a second target sequence and a second sequence primer, wherein said second hybridization complex is attached to a second microsphere;

d) simultaneously extending said first and second primers by the addition of a first nucleotide to a first detection position using a first enzyme to form first and second extended primer, respectively;

e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said first and second primers, respectively; and

f) determining the identity and location of each microsphere.

2. A method according to claim 1 wherein at least said first hybridization complex is covalently attached to said first microsphere.

3. A method according to claim 1 wherein at least said first sequencing primer is attached to said first microsphere.
4. A method according to claim 1 wherein said first and second hybridization complexes comprise:
 - a) said first and second target sequences;
 - b) said first and second sequencing primers;
 - c) first and second capture probes, wherein said capture probes are covalently attached to said first and second microspheres, respectively.
5. A method according to claim 1, wherein said first and second hybridization complexes comprise said first and second target sequences, respectively, said first and second sequencing primers, a first and second adapter probe, respectively, and first and second capture probes, respectively, covalently attached to said first and second microspheres.
6. A method according to claim 1 further comprising:
 - d) extending said first and second extended primers by the addition of a second nucleotide to a second detection position using said first enzyme; and
 - e) detecting the release of pyrophosphate (PPi) to determine the type of said second nucleotide added onto said first and second primers, respectively.
7. The method according to claim 1 wherein said PPi is detected by a method comprising:
 - a) contacting said PPi with a second enzyme that converts said PPi into ATP; and
 - b) detecting said ATP using a third enzyme.
8. A method according to claim 7 wherein said second enzyme is sulfurylase.
9. A method according to claim 7 wherein said third enzyme is luciferase.

11. A method according to claim 10 wherein said hybridization complex comprises said capture probe, an adapter probe, and said target sequence.
12. A method according to claim 10 wherein said sequencing primer is a capture probe.
13. A method according to claim 10 wherein said determining comprises:
 - a) providing a sequencing primer hybridized to said second domain;
 - b) extending said primer by the addition of a first nucleotide to a first detection position using a first enzyme to form an extended primer;
 - c) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer;
 - d) extending said primer by the addition of a second nucleotide to a second detection position using said enzyme; and
 - e) detecting the release of pyrophosphate (PPi) to determine the type of said first nucleotide added onto said primer.
14. The method according to claim 10 wherein said PPi is detected by a method comprising:
 - a) contacting said PPi with a second enzyme that converts said PPi into ATP; and
 - b) detecting said ATP using a third enzyme.
15. A method according to claim 14 wherein said second enzyme is sulfurylase.
16. A method according to claim 14 wherein said third enzyme is luciferase.
17. A method according to claim 10 wherein said determining comprises:
 - a) providing a sequence primer hybridized to said second domain;
 - b) extending said primer by the addition of a first protected nucleotide using a first enzyme to form an extended primer;

- c) determining the identification of said first protected nucleotide;
 - d) removing the protection group;
 - e) adding a second protected nucleotide using said first enzyme; and
 - f) determining the identification of said second protected nucleotide.
18. A kit for nucleic acid sequencing comprising:
- a) a composition comprising:
 - i) a substrate with a surface comprising discrete sites; and
 - ii) a population of microspheres distributed on said sites; wherein said microspheres comprise capture probes;
 - b) a first extension enzyme; and
 - c) dNTPs.
19. A kit according to claim 18 further comprising:
- d) a second enzyme for the conversion of pyrophosphate (PPi) to ATP; and
 - e) a third enzyme for the detection of ATP.
20. A kit according to claim 18 wherein said dNTPs are labeled.
21. A kit according to claim 20 wherein each dNTP comprises a different label.
22. The method according to claim 1, wherein said substrate comprises discrete sites and said first and second microspheres are randomly distributed on said sites.
23. The method according to claim 22, wherein said discrete sites are wells, and said first and second microspheres are randomly distributed in said wells.
24. The method according to claim 10, wherein said substrate comprises discrete sites and said microspheres are randomly distributed on said sites.

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25. The method according to claim 1, wherein discrete sites are wells, and said microspheres are randomly distributed in said wells.
26. The method according to claim 1, 10, 22, 23, 24 or 25, wherein said substrate is a fiber optic bundle.
27. The method according to claim 1, 22, 23, 24 or 25, wherein said substrate is selected from the group consisting of glass and plastic.
28. The kit according to claim 18, wherein discrete sites are wells.
29. The kit according to claim 18 or 28, wherein said substrate is a fiber optic bundle.
30. The kit according to claim 18 or 28, wherein said substrate is selected from the group consisting of glass and plastic.
- 31.(new) The method according to claim 1, wherein said microsphere array is decoded prior to providing first and second hybridization complexes.
32. (new) The method according to claim 31, wherein said microspheres further comprise an identifier binding ligand that will bind a decoder binding ligand such that the identity and location of each microsphere can be determined.